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No. of Pages: 18 including this Cover Sheet

Re: 0701.113C

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Fax No: 1-746-5010

Message:

#8 4-18-03
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0701.113C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Barberich et al.

Serial No.: 09/854,065 Group Art Unit: 1625

Filed: May 11, 2001 Examiner: Dentz, Bernard I.

Title: S-LANSOPRAZOLE COMPOSITIONS AND METHODS

INTERVIEW SUMMARY

To: Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This is a summary of an interview held in the office of Examiner Bernard Dentz at the USPTO on January 7, 2003. In addition to Examiner Dentz, those participating in the interview were Dr. Thomas R. Wagler, Director of Chemical Process R&D Outsourcing and Services at Sepracor Inc., Marlborough, Massachusetts; James C. Kellerman, Corporate Intellectual Property Counsel, Sepracor Inc., Marlborough, Massachusetts; and Philip E, Hansen, agent of record in this case.

Mr. Hansen opened the interview by outlining the present status of the case and explaining the purpose of the interview. The invention that is claimed in the application is a method of treating ulcers, GERD, gastric hypersecretion and psoriasis with S-(-)-lansoprazole. It is of considerable importance to Sepracor. Applicants hoped to convince the examiner that (1) the 102(b) rejection was inapposite and that (2) a *prima facie* case of obviousness had not been established for the 103(a) rejection.

The claims stand rejected over Larsson PCT application WO96/025353. Larsson describes the synthesis of S-(-)-lansoprazole by the tartrate catalyzed peroxidation of a thioether to a sulfoxide. The S-(-)-lansoprazole is produced as an oil. Elsewhere in the PCT application,

Larsson says that the single enantiomers produced by his method "may be converted to pharmaceutically acceptable salts" which may be "used in medicine". There is no actual description of the S-(-)-lansoprazole oil being converted to a salt or being administered to a human. Mr. Hansen urged that in light of the absence of any disclosure of S-(-)-lansoprazole actually being administered to a human, there was no anticipation of applicants' claim to a method of administering S-(-)-lansoprazole to a human. During a course of later discussion, both the Examiner and the applicants agreed that the studies that exist in the literature involve the administration of the racemic lansoprazole and the identification of individual enantiomers in the blood. Although there is a report of the activity of S-(-)-lansoprazole in isolated dog parietal cells, neither applicants nor the examiner are aware of any reports of the administration of S-(-)-lansoprazole to a whole animal. In light of this, Mr. Hansen urged that the rejection under 102 was inapposite.

Discussion then turned to the 103 rejection over von Unge PCT application WO97/02261. Von Unge's example 11 is a more detailed description of Larsson's example 21, which used the enrichment crystallization described in von Unge. Von Unge makes the further observation that "the single enantiomers of pharmacologically active compounds have met an increased interest in last years because of improved pharmacokinetic and biological properties." This is a general observation in keeping with the state of the art, but it imparts no special information about lansoprazole. Therefore, what von Unge adds to the general state of the art is that one can make each of the enantiomers of lansoprazole. Beyond that, there is only an invitation to experiment. To provide some perspective on why this invitation to experiment would not be perceived by the person of skill in the art as a motivation to separate the enantiomers, Mr. Hansen introduced Dr. Wagler.

Dr. Wagler outlined his background and expertise, then began by summarizing that developing a single enantiomer of a racemic drug for therapy is complicated and expensive.

Unless the person of skill expects a clear advantage, he/she would not be motivated to undertake the studies necessary to develop a single enantiomer. Dr. Wagler outlined the technological problems in general and those specific to S-(-)-lansoprazole.

Several techniques are available for obtaining single enantiomers of chiral molecules. Techniques that begin from a racemic mixture of isomers include physical separation by chromatography, classical separation by crystallization and enzymatic resolution. Separation of a racemate is usually unattractive because 50% of the very valuable racemic material is wasted. Single enantiomers may also be obtained by synthesis, either by beginning with a compound that is available naturally as a single isomer or by asymmetric synthesis using a reagent that imparts chirality. In theory, synthetic methods are more attractive than separation because one can, in principle, obtain yields above 50%.

Chiral chromatography is generally not attractive on more than milligram scale. Yields above 45% are seldom encountered; the cost of the stationary phase is usually quite high and the stationary phase has a limited useful life. Chromatography on a scale for clinical studies requires enormous amounts of solvent, which, in the case of organic solvents leads to high initial cost and to waste disposal problems. Separation is limited by the ability of available stationary phases to resolve the particular isomers of interest, and there is usually a trade-off between enantiomeric excess and yield. Finally, chromatography as a final step of a synthesis of an active drug principal presents problems because of the impurities that commonly bleed off columns after repeated or prolonged use.

Classical resolution by diastereomeric salts, like chiral chromatography, limits the maximum yield to a theoretical 50%. In practice, 50% yield is never achieved in a simple resolution without recycling the undesired isomer through a racemization process. The method is further limited in that only acidic or basic materials can be resolved by their diastereomeric salts.

Lansoprazole presents particular problems in this regard. The compound is basic, but exposing it to acid to form a salt induces a rearrangement of the sulfoxide to form an achiral pyridinium sulfenamide. The skilled artisan would expect that finding an appropriate acid to resolve lansoprazole would be difficult at best, and there is no guarantee it is even possible. The problem has been partly circumvented in the case of omeprazole by preparing a neutral diastereomeric aminoacetal and carrying out the resolution on the aminoacetal, then hydrolyzing the acetal. However, this method suffers from some very serious disadvantages.

PCT application WO 97/02261 (von Unge) describes a variation on the crystallization theme. In this case, a non-racemic mixture of the isomers of lansoprazole is crystallized from acetonitrile. The racemate is the least soluble component and crystallizes out preferentially, leaving a mother liquor substantially enriched in whichever enantiomer predominated in the non-racemic mixture. This is also not a practical process. It requires a synthetic route to a non-racemic mixture of enantiomers; it provides poor yields from a very dear starting material; and the product still has to be crystallized in a subsequent step.

Enzymatic resolution is described in PCT application WO 96/17077 (Graham) for producing single enantiomers of sulfoxide proton pump inhibitors. The enantiomeric excess was only 58%, and the reaction had to be carried out at a substrate concentration of 0.1 g per liter (0.01%). The low concentration leads to expensive, complex recovery processes, large amounts of waste to dispose of and, in the case of lansoprazole, a product of very low optical purity.

Synthesis *de-novo* from the chiral pool available in nature is limited to compounds that can be derived by a convenient synthesis from cheaply available natural starting materials. There are no such syntheses or starting materials for any proton pump inhibitors.

Asymmetric synthesis is generally the most attractive route for obtaining single enantiomers of synthetic materials. PCT application WO 96/02535 and its US counterpart, US patent 5,948,789, describe a chiral oxidation of the sulfide precursor of lansoprazole to lansoprazole. The process provides a 29% yield of S-lansoprazole, as an oil, after chromatography and repeated crystallization from acetonitrile. It would still have to be processed further to provide a medicament that could be administered to humans.

At this point, there was a brief discussion in which Examiner Dentz inquired about how the applicants make S-(-)-lansoprazole and whether they could make a showing. Mr. Kellerman indicated that clinical trials to make the showing are very difficult, time-consuming and expensive. Applicants have reason to believe that S-(-)-lansoprazole is superior to the racemate, but there are no results of clinical trials. At present, applicants must rely on their assertion that no *prima facie* case of obviousness has been made.

Mr. Hansen summarized applicants' presentation. While the pure enantiomers of lansoprazole are known, there are no good processes for producing them on a scale suitable for assessing the possible clinical advantages of one enantiomer. This is a very high motivational hurdle for the person of skill contemplating whether to try such experiments.

Under other circumstances and relating to other racemic drugs where nothing is known about the individual enantiomers, the question of motivation of the skilled artisan to try the claimed invention might be more equivocal. Lansoprazole, however, is not a case where the literature was silent, and where the person of skill might or might not be motivated to explore the properties of the enantiomers. Here, the literature expressly taught the complete therapeutic equivalence of the two enantiomers. At the time of applicants' filing, the person of skill "knew" from the literature that experiments to demonstrate an advantage of one or the other enantiomer would fail! This is a case where the person of skill clearly would have a very high hurdle (as

explained by Dr. Wagler) and no motivation. Applicants urge that, under those circumstances, a *prima facie* case of obviousness does not exist.

Dr Wagler, Mr. Kellerman and Mr. Hansen thanked Examiner Dentz for his time and attention and the interview was concluded.

Respectfully submitted,

Philip E. Hansen
Agent for Applicants
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Dated: January 13, 2003

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J. Pharm. Pharmacol. 1998, 50: 1241–1245
Received May 11, 1998
Accepted July 5, 1998



# Pharmacokinetic Differences Between Lansoprazole Enantiomers in Rats

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**Abstract**

Because limited information is available about potential differences between the pharmacokinetics and pharmacodynamics of the enantiomers of lansoprazole, the enantioselective pharmacokinetics of the compound have been investigated in rats.

There was a noticeable difference between the serum levels of the enantiomers of lansoprazole and of their metabolites, 5-hydroxylansoprazole enantiomers, after oral administration of the racemate (50 mg $kg^{-1}$) to rats. $C_{max}$ (maximum serum concentration) and AUC (area under the serum concentration–time curve) for (+)-lansoprazole were 5–6 times greater than those for (−)-lansoprazole, whereas for (+)-5-hydroxylansoprazole both values were significantly smaller than those for the (−) enantiomer. $CL_{tot}/F$ values (where $CL_{tot}$ is total clearance and F is the fraction of the dose absorbed) for (+)-lansoprazole were significantly smaller than those for the (−) enantiomer. There was no significant difference between the absorption rate constants of the lansoprazole enantiomers in the in-situ absorption study. The in-vitro protein-binding study showed that binding of (+)-lansoprazole to rat serum proteins was significantly greater than for the (−) enantiomer. The in vitro metabolic study showed that the mean metabolic ratio (45·9%) for (−)-lansoprazole was significantly greater than that (19·8%) for the (+) enantiomer in rat liver microsomes at 5·6 μM lansoprazole.

These results show that the enantioselective disposition of lansoprazole could be a consequence of the enantioselectivity of plasma–protein binding and the hepatic metabolism of the enantiomers.

Lansoprazole is a benzimidazole derivative which powerfully and continuously inhibits gastric proton-pump ($H^+/K^+$ ATPase) activity in the final step of gastric acid secretion in the parietal cells (Wallmark et al 1983). The drug is extensively metabolized in the liver and the major metabolites present in the plasma are 5-hydroxylansoprazole and lansoprazole sulphone. Formation of the 5-hydroxy metabolite is mediated by cytochrome P450 2C19 (CYP2C19), whereas the formation of the sulphone is mediated by CYP3A4 (Pichard et al 1995; Sohn et al 1997). Lansoprazole has an asymmetric sulphur in the chemical structure (Figure 1) and is administered clinically as a racemic mixture of the (+) and (−) enantiomers. Racemic lansoprazole is metabolized in the liver to (+)- and (−)-5-hydroxylansoprazole; the sulphone metabolite is achiral. Because limited information is available about potential differences between the pharmacokinetics and pharmaco-dynamics of the enantiomers of lansoprazole (Miwa et al 1990; Nagaya et al 1991; Katsuki et al 1996), it is important to evaluate the pharmacokinetics of the individual enantiomers because the pharmacological effects or toxicity, or both, of the enantiomers might be different. On the stereoselective pharmacokinetics of lansoprazole, there is only our report on the pharmacokinetics of the enantiomers in healthy subjects using a chiral stationary phase column, Chiralpak AS (amylose tris(S-1-phenylethylcarbamate)) for HPLC determination (Katsuki et al 1996).

This study extends our previous work on stereoselective differences between the pharmacokinetic behaviour, i.e. absorption, metabolism and protein binding, of the enantiomers of lansoprazole in rats.

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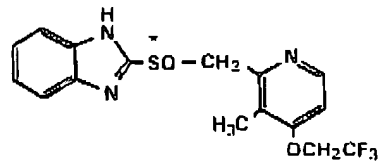
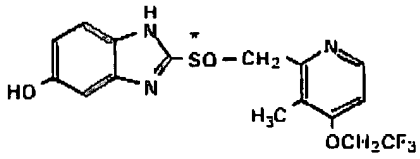
## Materials and Methods

### *Materials*

Racemic lansoprazole and its metabolite 5-hydroxy lansoprazole were kindly supplied by Takeda




Lansoprazole




5-Hydroxylansoprazole

Figure 1. Chemical structures of lansoprazole and 5-hydroxy lansoprazole. *Asymmetric centre

Chemical Industries (Osaka, Japan). Other chemicals used in this study were of analytical grade.

*In-vivo experiments*

Male Wistar rats, 250–320 g, were fasted overnight with free access to water. Under light ether anaesthesia, lansoprazole (50 mg $kg^{-1}$) was administered orally as 1 mL of a suspension in 0·5% methylcellulose containing 0·2% $NaHCO_3$ adjusted to pH 9 with 0·1 N NaOH. Blood samples (300 $\mu$L) were collected periodically from a cut at the tip of the tail.

*In-situ absorption study*

The rats were anaesthetized by intraperitoneal injection of ethyl carbamate (urethane; 1·2 g $kg^{-1}$). The small intestine was exposed by midline abdominal incision and the upper duodenum and the ileocaecal junction were cannulated with polyethylene tubing. Intestinal absorption experiments were performed by a conventional in-situ recirculation method (Tominaru et al 1996). Lactated Ringer's solution (pH 6·5, maintained at 37°C; 100 mL) containing lansoprazole (50 $\mu$g $mL^{-1}$) was perfused from the duodenum through the small intestine to the ileocaecal junction at a rate of 5 mL $min^{-1}$. Perfusates were collected 0 and 60 min after the start of the experiment. The absorption rate constant ($k_a$) was obtained by use of the equation:

$$k_a = \ln(C_0V_0/C_1V_1)/t \qquad (1)$$

where $t$ is the perfusion time, $C_0$ and $C_1$ are the concentrations of lansoprazole in the perfusates at $t = 0$ and $t = 60$ min, respectively, and $V_0$ and $V_1$ are the volume of the perfusates at $t = 0$ and $t = 60$ min, respectively.

*Protein-binding study*

Protein-binding experiments were performed in triplicate by means of an ultrafiltration technique using Centrifree MPS-3 (Amicon, Danvers, MA) as reported previously (Arimori & Nakano 1987). Briefly, serum samples (1 mL) containing added racemic lansoprazole (6·3 $\mu$g $mL^{-1}$) were incubated for 30 min at 37°C. After incubation, the samples were ultrafiltered at 1000 $g$ for 20 min at 4°C. The fraction of unbound drug was determined by use of the equation:

$$f_u = C_u/C_t \qquad (2)$$

where $f_u$ is the fraction of unbound drug in the serum and $C_u$ and $C_t$ are, respectively, the concentration of unbound drug and the total concentration of the drug in the serum.

*Preparation of rat liver microsomes*

The liver microsomes were prepared from male Wistar rats according to a method reported elsewhere (Tsuruta et al 1997). All subsequent procedures were performed at 4°C or lower. After determination of protein concentration by the method of Lowry et al (1951), a microsomal suspension was prepared at a concentration of 1–2 mg $mL^{-1}$ and was kept at −80°C until used.

*Determination of metabolic ratio*

Metabolic ratio is defined as a ratio of the amount of each enantiomer of lansoprazole eliminated to the amount of each enantiomer added, part of which was metabolized by microsomal enzymes. The reaction medium contained microsomes (0·1 mg $mL^{-1}$; 100 $\mu$L), potassium phosphate buffer (0·3 mM, pH 7·4; 200 $\mu$L), EDTA (0·6 mM; 100 $\mu$L) and lansoprazole (5·6 $\mu$M). The mixture was pre-incubated at 37°C for 5 min and subsequently at 37°C for 30 min after addition of NADPH-generating system ($NADP^+$, 3 mM; glucose 6-phosphate, 12 mM; glucose-6-phosphate dehydrogenase, 6 international units $mL^{-1}$; $MgCl_2$, 24 mM; 100 $\mu$L). The reaction was stopped by adding 3 mL of 7 : 3 (v/v) diethyl ether–dichloromethane.

*Isolation and determination of lansoprazole*

Isolation of racemic lansoprazole and determination of lansoprazole enantiomers in serum were performed by high-performance liquid chromatography (HPLC) as reported elsewhere (Katsuki et al 1996). The isolation was performed on a 250 mm × 4·0 mm i.d., 5 $\mu$m particle LiChrospher

100 RP-18(e) reversed phase column. The mobile phase was 35:65 acetonitrile–water adjusted to pH 7·0 with phosphoric acid and containing 0·1% *n*-octylamine. The eluate, flow rate 1·0 mL $min^{-1}$, was monitored for absorbance at 285 nm and the portion eluting between 2 min before and 2 min after the peak of racemic lansoprazole was collected. The residue from this fraction was reconstituted in 8:2 (v/v) *n*-hexane–ethanol (200 $\mu$L) and 100 $\mu$L of this solution was injected on to a chiral HPLC column for separation of the enantiomers.

Determination of lansoprazole enantiomers in serum was performed by normal-phase HPLC on a 25 cm × 4·6 mm i.d. Chiralpak AS column (Daicel, Tokyo, Japan). The HPLC mobile phase was 8:2 (v/v) *n*-hexane–ethanol; the flow rate was 1·0 mL $min^{-1}$. The analytical column was maintained at 38°C.

*Pharmacokinetic analysis*

Serum concentration–time curves were analysed by non-linear regression analysis using a one-compartment model. The maximum serum concentration ($C_{max}$) and the time required to reach $C_{max}$ ($t_{max}$) were obtained graphically. The plasma concentrations of the elimination phase were used to calculate the elimination rate constant ($k_{el}$) by exponential regression analysis. The areas under the concentration–time curves ($AUC_{0-t}$) were calculated by a trapezoidal rule and by extrapolating time to infinity by use of $k_{el}$ values. The terminal half-life ($t_{\frac{1}{2}}$) was calculated by dividing 0·693 by $k_{el}$. The apparent total body clearance ($CL_{tot}/F$) was calculated from $CL_{tot}/F = dose/AUC_{0-\infty}$, F being the fraction of the dose absorbed.

*Statistical analysis*

Results are expressed as means + s.e.m. Differences between pharmacokinetic data were analysed for statistical significance by use of Student's *t*-test. A probability level of $P < 0.05$ was considered to be indicative of significance.

## Results

*In-vivo study*

Figure 2 shows the serum concentration–time profiles of the enantiomers of lansoprazole and its metabolite 5-hydroxylansoprazole after oral administration of racemic lansoprazole (50 mg $kg^{-1}$) to rats. There was a noticeable difference between the serum levels of the enantiomers both of lansoprazole and of 5-hydroxylansoprazole. The mean serum levels of (+) lansoprazole were higher at all time-points than those of (−)-lansoprazole during the experimental period and the mean serum levels of the metabolite, (+)-5-hydroxylansoprazole were lower than those of (−)-5-hydroxylansoprazole. The pharmacokinetic parameters are summarized in Table 1. $C_{max}$ and $AUC_{0-6}$ values for (+)-lansoprazole were 5–6 times greater than those for the (−) enantiomer ($P < 0.01$) and $CL_{tot}/F$ values for (+)-lansoprazole were significantly smaller than those for the (−) enantiomer ($P < 0.01$). $C_{max}$ and $AUC_{0-6}$ for (+)-5-hydroxylansoprazole were significantly smaller than those for (−)-5-hydroxylansoprazole. There was no significant difference between the $t_{max}$ values of the enantiomers.

*In situ absorption study*

We investigated enantioselectivity in the intestinal absorption of lansoprazole by the in-situ recirculation

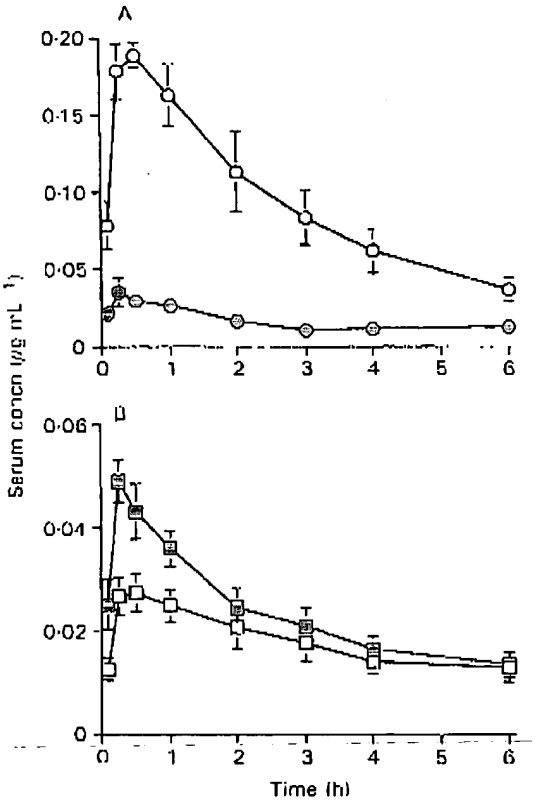



Figure 2. Serum concentration–time profiles of the enantiomers of lansoprazole (A) and 5-hydroxylansoprazole (B) after oral administration of racemic lansoprazole (50 mg $kg^{-1}$) to rats. ○, (+)-lansoprazole; ●, (−)-lansoprazole; □, (+)-5-hydroxylansoprazole; ■, (−)-5-hydroxylansoprazole. Each point and bar represent the mean ± s.e.m. of results from six rats.

Table 1. Pharmacokinetic parameters of lansoprazole and 5-hydroxylansoprazole enantiomers in rats.

| | $AUC_{0-6}$ (μg h mL$^{-1}$) | $C_{max}$ (μg mL$^{-1}$) | $t_{max}$ (h) | $t_{\frac{1}{2}}$ (h) | $CL_{tot}/F$ (L h$^{-1}$ kg$^{-1}$) |
|---|---|---|---|---|---|
| (+)-Lansoprazole | 0·549 ± 0·087 | 0·194 ± 0·012 | 0·458 ± 0·119 | 1·88 + 0·32 | 90·7 + 18·8 |
| (−)-Lansoprazole | 0·088 ± 0·015† | 0·037 ± 0·008† | 0·500 + 0·112 | 2·62 ± 0·97 | 437·0 ± 62·5† |
| (+)-5-Hydroxylansoprazole | 0·105 + 0·019 | 0·029 ± 0·035 | 0·375 ± 0·056 | 3·79 + 0·52 | |
| (−)-5-Hydroxylansoprazole | 0·140 + 0·017* | 0·049 + 0·004† | 0·417 ± 0·124 | 2·56 ± 0·33 | |

Each value is the mean ± s.e.m. of results from six rats. *$P < 0·05$, †$P < 0·01$. $AUC_{0-6}$ area under the serum concentration–time curve between 0 and 6 h; $C_{max}$, maximum serum concentration; $t_{max}$, time of maximum serum concentration; $t_{\frac{1}{2}}$, terminal half-life; $CL_{tot}$, total clearance; F, fraction of the dose absorbed; $CL_{tot}/F$, apparent total body clearance.

Table 2. Stereoselective protein binding and metabolism of (+)- and (−)-lansoprazole in-vitro.

| | Unbound fraction (%) | Metabolic ratio (%) |
|---|---|---|
| (+)-Lansoprazole | 1·2 ± 0·7 | 19·8 ± 3·4 |
| (−)-Lansoprazole | 4·8 ± 0·3* | 45·9 ± 2·5† |

Each value is the mean ± s.e.m. of results from four (protein-binding study) or three (metabolism study) rats. *$P < 0·05$. †$P < 0·01$.

method. There was no significant difference between the absorption of the lansoprazole enantiomers. The average absorption rate constants ($k_a$) of (+)- and (−)-lansoprazole were 0·64 and 0·69 h$^{-1}$ (data not shown).

*In-vitro protein-binding study*

The extent of enantioselective binding of lansoprazole to rat serum was estimated by an ultrafiltration technique. The binding of (+)-lansoprazole to rat serum was significantly greater than that of the (−) enantiomer (Table 2). The mean unbound fractions of the (+) and (−) enantiomers were 1·2 and 4·8%, respectively.

*In-vitro metabolic study*

The enantioselective metabolism of lansoprazole by rat-liver microsomes was investigated at a concentration of 5·6 μM. The mean metabolic ratio (45·9%) of the (−) enantiomer was significantly greater than that (19·8%) of the (+) enantiomer in rat liver microsomes (Table 2).

## Discussion

We have investigated the stereoselectivity of the pharmacokinetics of lansoprazole in rats after oral administration of racemic lansoprazole as a suspension (pH 9). The $C_{max}$ and $AUC_{0-6}$ values of (+)-lansoprazole were markedly greater than those of (−)-lansoprazole (Figure 2 and Table 1), implying stereoselective absorption, distribution, metabolism or excretion of the drug.

Although absorption of both enantiomers from the small intestine was relatively rapid and their $t_{max}$ values were close there was a pronounced difference between the $C_{max}$ values of the enantiomers (the ratio $C_{max}$ (+)/$C_{max}$ (−) was 5·3). We examined whether or not lansoprazole enantiomers are absorbed enantioselectively. The in-situ absorption study showed no evidence of stereoselective absorption of lansoprazole from the intestine.

The extent of binding of enantiomers to plasma proteins is an important factor in tissue distribution because only unbound drugs can permeate biomembranes. In-vitro and in-vivo experiments have shown that lansoprazole is 91–96% bound to albumin in the rat (Miwa et al 1990). In the current study the extent of protein binding of (+)-lansoprazole was significantly greater than that of (−)-lansoprazole (Table 2). Therefore, (+)-lansoprazole which is more extensively bound to serum proteins could be poorly distributed and slowly metabolized, resulting in the serum concentrations higher than those of (−)-lansoprazole. Consequently, enantioselective protein binding might influence the enantioselective disposition of lansoprazole after oral administration.

There is a possibility of stereoselectivity in the liver metabolism of lansoprazole enantiomers as reported for other proton-pump inhibitors (Uematsu et al 1994; Tybring et al 1997). Lansoprazole is metabolized extensively by the liver and its primary metabolite in the serum is 5-hydroxylansoprazole, which is also chiral, and lansoprazole sulphone, with no recovery of the unchanged drug in the urine (Tateno & Nakamura 1991). Our results showed that the $C_{max}$ value of (−)-5-hydroxylansoprazole was significantly greater than that of (+)-5-hydroxylansoprazole (Figure 2). In addition, the metabolic ratio of (−)-lansoprazole was 2·3 times greater than that of (+)-lansoprazole in rat liver microsomes (Table 2). These results

confirm that metabolism of lansoprazole enantiomers in the liver is enantioselective. Possible chiral inversion between lansoprazole enantiomers in the liver and intestine has not yet been studied. Jeffrey et al (1991) reported that inversion of *R*-(−)-ibuprofen to the *S*-(−) antipode occurred in the rat liver. In the current study we did not examine possible inversion because of the unavailability of adequate amounts of the enantiomers.

The metabolism of lansoprazole has been reported to be dependent on CYP2C19 activity for 5-hydroxylation of the drug (Pichard et al 1995). We also reported that subjects with genetic defects of CYP2C19 (m1/m1 and m1/m2) had lower $C_{max}$ values for 5-hydroxylansoprazole than subjects with homozygous wild-type (wt/wt) and heterozygote (wt/m1 and wt/m2) (Katsuki et al 1997). Thus the pronounced enantioselective metabolism of the enantiomers of lansoprazole could be a consequence of different affinities for CYP2C19 isozyme. Omeprazole, inhibitor of the same proton pump as lansoprazole, is also metabolized to 5-hydroxyomeprazole mainly by CYP2C19 (Andersson et al 1990). Tybring et al (1997) reported that $AUC_{0-8}$ values for (+)-omeprazole in the poor metabolizers phenotyped for CYP2C19 were 7.5-fold those for the extensive metabolizers, whereas the $AUC_{0-8}$ values for (−)-omeprazole in the poor metabolizers were only 3.1-fold those in the extensive metabolizers. They suggested that (+)-omeprazole is to a major extent hydroxylated by CYP2C19, whereas (−)-omeprazole might be metabolized partly by this enzyme but mainly by another, presumably CYP3A4, to the achiral sulphone metabolite. This might support the hypothesis that the affinities of the lansoprazole enantiomers for CYP2C19 isozyme are different. Further studies are needed to elucidate to what extent each enantiomer is catalysed by other isozymes in addition to CYP2C19.

In conclusion, it was confirmed that protein binding and metabolism of lansoprazole in the liver are enantioselective and that this is responsible for the different pharmacokinetics of the enantiomers of lansoprazole.

## References


Andersson, T., Regardh, C. G., Dahl-Puustinen, M. L., Bertilsson, L. (1990) Slow omeprazole metabolizers are also poor *S*-mephenytoin hydroxylators. Ther. Drug Monit. 12: 415–416

Arimori, K., Nakano, M. (1987) The intestinal dialysis of intravenously administered phenytoin by oral activated charcoal in rats. J. Pharmacobiodyn. 10: 157–165

Jeffrey, P., Tucker, G. T., Bye, A., Crewe, H. K., Wright, P. A. (1991) The site of inversion of *R*-(−)-ibuprofen: studies using rat in-situ isolated perfused intestine/liver preparations. J. Pharm. Pharmacol. 43: 715–720

Katsuki, H., Yagi, H., Arimori, K., Nakamura, C., Nakano, M., Kanafuchi, S., Fujioka, Y., Fujiyama, S. (1996) Determination of *R*-(+)- and *S*-(−)-lansoprazole using chiral stationary-phase liquid chromatography and their enantioselective pharmacokinetics in humans. Pharm. Res. 13: 611–615

Katsuki, H., Nakamura, C., Arimori, K., Fujiyama, S., Nakano, M. (1997) Genetic polymorphism of CYP2C19 and lansoprazole pharmacokinetics in Japanese subjects. Eur. J. Clin. Pharmacol. 52: 391–396

Lowry, O. H., Rosebrough, A. L., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Miwa, K., Murani, M., Tsukamoto, T., Yoshida, K., Kobayashi, T., Kimura, T., Simomura, H., Tanayama, S. (1990) Metabolic fate of AG-1749, a new proton pump inhibitor, in rats, mice, and dogs. Jpn Pharmacol. Ther. 18: 3413–3435

Nagaya, H., Inatomi, N., Nohara, A., Satoh, H. (1991) Effects of the enantiomers of lansoprazole (AG-1749) on ($H^+$+$K^+$)-ATPase activity in canine gastric microsomes and acid formation in isolated canine parietal cells. Biochem. Pharmacol. 42: 1875–1878

Pichard, L., Curi-Pedrosa, R., Bonfils, C., Jacqz-Aigrain, E., Domergue, J., Joyeux, H., Cosme, J., Guengerich, F. P., Maurel, P. (1995) Oxidative metabolism of lansoprazole by human liver microsomes. Mol. Pharmacol. 47: 410–418

Sohn, D. R., Kwon, J. T., Kim, H. K., Ishizaki, T. (1997) Metabolic disposition of lansoprazole in relation to the *S*-mephenytoin 4′-hydroxylation phenotype status. Clin. Pharmacol. Ther. 61: 574–582

Tateno, M., Nakamura, N. (1991) Phase I study of lansoprazole (AG-1749) antiulcer agent-Capsule form. Rinsho Iyaku 7: 51–62

Tomimari, A., Arimori, K., Inotsume, N., Nakano, M. (1996) Effect of activated charcoal and atropine on absorption and/or excretion of organophosphorus compounds in rats. J. Pharm. Pharmacol. 48: 351–356

Tsuruta, S., Nakamura, K., Arimori, K., Nakano, M. (1997) Effects of erythromycin, clarithromycin and rokitamycin on nifedipine metabolism in rats. Biol. Pharm. Bull. 20: 411–416

Tybring, G., Bottiger, Y., Widen, J., Bertilsson, L. (1997) Enantioselective hydroxylation of omeprazole catalyzed by CYP2C19 in Swedish white subjects. Clin. Pharmacol. Ther. 62: 129–137

Uematsu, T., Nakano, M., Kosuge, K., Nagai, A., Sato, A., Nakashima, M. (1994) Pharmacokinetic properties of a novel gastric proton-pump inhibitor, (±)-2-[(4-methoxy-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridin-9-yl)sulfinyl-1*H*-benzimidazole sodium salt, in healthy subjects. J. Pharm. Sci. 83: 1407–1411

Wallmark, B., Jaresten, B. M., Larsson, H., Ryberg, B., Brandstrom, A., Fellenius, E. (1983) Differentiation among inhibitory actions of omeprazole, cimetidine and SCN on gastric acid secretion. Am. J. Physiol. 245: G64–G71

0701.113C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Barberich et al.

Serial No.: 09/854,065 Group Art Unit: 1625

Filed: May 11, 2001 Examiner: Dentz, Bernard J.

Title: S-LANSOPRAZOLE COMPOSITIONS AND METHODS

AFFIDAVIT UNDER 37 C.F.R. §1.132

To: Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Thomas R. Wagler, being duly sworn does depose and say as follows:

1. I reside at 5 Hemingway Street, Shrewsbury, Massachusetts, 01545;

2. I earned a B.A. in Economics and Business Administration from Ursinus College in 1982, and a Ph.D. degree in Synthethic Organic Chemistry from the State University of New York at Stony Brook in 1988. My primary area of responsibility for the past 12 years has been in commercial synthetic processes and process scale-up. I am presently Director – Chemical Process R&D Outsourcing and Services at Sepracor Inc., Marlborough, Massachusetts. Prior to my employment at Sepracor, I was (sequentially) Senior Research Investigator at Bristol-Myers Squibb, Lawrenceville, New Jersey; Project Team Leader – Process Research Group at Intercardia Pharmaceuticals, Cranbury, New Jersey; and Group Leader – Process R&D at FMC Corp, Princeton, New Jersey.

3. I am a member of the American Chemical Society and the Synthetic Organic Chemical Manufacturers' Association (SOCMA);

4. I am the author of approximately 11 papers in the area of pharmaceutical synthesis and an inventor in four U.S. patents.

5. I have reviewed and do understand the contents of the above-identified application, which is directed to methods employing pure S-(-)-lansoprazole for the treatment of ulcers in humans. I have also reviewed the Office Action in the present case, dated April 19, 2002, as well as the references cited therein: Von Unge PCT Application WO 97/02261 and Larsson PCT Application WO 96/02535.

In support of the nonobviousness of the method and compositions claimed in the above application, I present herewith my opinion as an expert in the area of process development:

Summary

Developing a single enantiomer of a racemic drug for therapy is complicated and expensive. Unless the person of skill expects a clear advantage, he/she would not be motivated to undertake the studies necessary to develop a single enantiomer.

Discussion

Several techniques are available for obtaining single enantiomers of chiral molecules. Techniques that begin from a racemic mixture of isomers include physical separation by chromatography, classical separation by crystallization and enzymatic resolution. Separation of a racemate is usually unattractive because 50% of the very valuable racemic material is wasted. Single enantiomers may also be obtained by synthesis, either by beginning with a compound that is available naturally as a single isomer or by asymmetric synthesis using a reagent that imparts chirality. In theory, synthetic methods are more attractive than separation because one can, in principle, obtain yields above 50%. The following observations may be made with respect with each of these techniques:

Chiral chromatography is generally not attractive on a scale that would be adequate to conduct a clinical trial. The maximum theoretical yield is 50%, and yields above 45% are seldom encountered. The cost of the stationary phase is usually quite high and the stationary phase has a limited useful life. Chromatography on large scale requires enormous amounts of solvent, which, in the case of organic solvents leads to high initial cost and to waste disposal problems. Separation is limited by the ability of available stationary phases to resolve the particular isomers of interest, and there is usually a trade-off between enantiomeric excess and yield. Finally, chromatography as a final step of a synthesis of an active drug principal presents problems because of the impurities that commonly bleed off columns after repeated or prolonged use.

Classical resolution by diastereomeric salts, like chiral chromatography, limits the maximum yield to a theoretical 50%. In practice, 50% yield is never achieved in a simple resolution without recycling the undesired isomer through a racemization process. The method is further limited in that only acidic or basic materials can be resolved by their diastereomeric salts. Lansoprazole presents particular problems in this regard. The compound is basic, but exposing it to acid to form a salt induces a rearrangement of the sulfoxide to form an achiral pyridinium sulfenamide. The skilled artisan would expect that finding an appropriate acid to resolve lansoprazole would be difficult at best, and there is no guarantee it is even possible. The problem has been partly circumvented in the case of omeprazole by preparing a neutral diastereomeric aminoacetal and carrying out the resolution on the aminoacetal, then hydrolyzing the acetal. However, as stated in PCT WO 97/02261, this method suffers from at least three fundamental disadvantages:

1. The substituted 2-(2-pyridinylmethylsulphinyl)-1H-benzimidazole, as a racemic intermediate, has to be further processed in a couple of reaction steps before the single enantiomers can be obtained.

2. The resolution processes involve complicated separation steps.

3. There is a large waste of highly refined material when the unwanted stereoisomer, in the form of the opposite diastereomer, is discarded.

PCT application WO 97/02261 (von Unge) describes a variation on the crystallization theme. In this case, a non-racemic mixture of the isomers of lansoprazole is crystallized from acetonitrile. The racemate is the least soluble component and crystallizes out preferentially, leaving a mother liquor substantially enriched in whichever enantiomer predominated in the non-racemic mixture. This is an unattractive process for at least three reasons: (1) It requires a synthetic route to a non-racemic mixture of enantiomers. (2) In the example given (Example 11) 1.2 g of non-racemic lansoprazole provided 0.63 g of S-lansoprazole. Thus the yield from a very dear starting material was 53%. (3) Finally, the 99% optically pure product is provided as an oil. It still has to be crystallized in a subsequent step - with the attendant further loss of material.

Enzymatic resolution is described in PCT application WO 96/17077 (Graham) for producing single enantiomers of sulfoxide proton pump inhibitors. Table 7 (page 19) discloses that lansoprazole was bioreduced in 96% yield (i.e. 48 mg from 100 mg of racemate), but the enantiomeric excess was only 58%. The reaction was carried out at a substrate concentration of 0.1 g per liter (0.01%). This process illustrates two of the major problems of enzymatic resolution: enzymes are highly substrate specific; finding an enzyme that converts a given synthetic substrate in high e.e. is very much hit-or-miss; and the incubation must usually be carried out at an unattractively low concentration. This leads to expensive, complex recovery processes, large amounts of waste to dispose of and, in the case of lansoprazole, a product of very low optical purity.

Synthesis *de novo* from the chiral pool available in nature is limited to compounds that can be derived by a convenient synthesis from cheaply available natural starting materials. There

are no such syntheses or starting materials for any proton pump inhibitors. Presumably this is because naturally available materials having chirality at a sulfoxide bond are extremely rare.

Asymmetric synthesis is generally the most attractive route for obtaining single enantiomers of synthetic materials. PCT application WO 96/02535 and its US counterpart, US patent 5,948,789, describe a chiral oxidation of the sulfide precursor of lansoprazole to lansoprazole. The process begins with 2.1g of sulfide and ends up with 0.63 g (29%) of S-lansoprazole, as an oil, after chromatography and repeated crystallization from acetonitrile (see above). Racemic lansoprazole is a solid having a melting point of 149-150°C (US patent 5,045,321, column 12, line 19). This suggests that the material recovered in 29% yield after an arduous process is still not chemically pure. Moreover, since oils are not readily formulated for dosage forms, one would want to crystallize the oil in some fashion to prepare a medicament, even if it were pure. One of skill would conclude that, like many asymmetric syntheses, the synthetic process described in the '789 patent might be serviceable for academic purposes, but one would only consider it as a starting point for a practical process if there were some compelling advantage to the use of a single enantiomer.

In summary, there are no good processes for producing either pure enantiomer of lansoprazole if one is envisioning its use in human therapy. The literature at the time of the applicants' invention indicated no therapeutic advantage to either enantiomer. More importantly,

the literature expressly taught the complete therapeutic equivalence of the two enantiomers. In the face of this, the person of skill in the art would not be motivated to attempt to separate the enantiomers for the claimed purpose of administering a single pure enantiomer to a human.

Thomas R. Wagles Ph.D

Affiant

Subscribed and sworn to before
me this day of January 2003

Notary Public